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RESEARCH ARTICLE

Anticancer Activity of the *in Vitro*-Derived Extract of Cactus (*Opuntia ficus-indica L. Mill*) Treated with Selenium Nanoparticles

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ABSTRACT: The anticancer activity of the extracts produced from *in-vitro* cladodes of *Opuntia ficus-Indica* L. Mill was evaluated. In which, two different treatments of selenium nanoparticles (SeNPs) 2.5 and 5 mg were supplemented to the basal culture medium. The highest number of shoots per explant and the maximum length of *Opuntia* shoots were obtained with selenium nanoparticles (5 mg/l) at 11.33 shoots/explant and 8.20 cm, respectively. The highest total phenolic content was found at 168.5 mg/g in the extract of *in-vitro* cladodes, which were previously cultured on MS medium provided with selenium nanoparticles at 5.0 mg/l, compared to the extract of *in-vitro* cladodes cultured on MS basal medium with selenium nanoparticles at 2.5 mg/l,which recorded 91.5 mg/g DW of total phenolic. These significant results revealed anticancer activity on both the viability and proliferation/migration of A549 lung cells. Considering cytotoxicity, the IC50 value on A549 cells was 3535.92 µg/ml, showing dose-dependent increases. Meantime, from the in vitro control analyses of the plants, the IC50 value was 3766.16 µg/ml, again showing an increase based on dose dependence. These concluded results refer to a new agent for lung cancer prevention instead of chemoprevention.

KEYWORDS: Nano selenium (SeNPs), Preakly pear cactus, Anti-cancer activity

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1. INTRODUCTION

Selenium (Se) is one of the trace minerals that are substantial for the maintenance of plant physiology and having in the organic Se forms in different parts of *Opuntia* such as fruits, cladode, and seeds could improve the health and increase Se mineral nutrition, furthermore, Se forms have a role to support the prevention of multiple human cancers [1]. Recently, several researchers have studied the role of selenium nanoparticles (SeNPs) and their applications in medicine, pharmaceuticals, and agriculture conducting plenty of research to study the effects of SeNPs on plant physiology and nutrition. Regarding various biological processes and specifically the yield in the soil and plants, showing the comparison between the results of applying Se in standard fertilizer form and Nano form SeNPs shows that Se in standard fertilizer form is considered less efficient than using SeNPs in the standard fertilizer form [2]. Nowadays, the number of applied patents and nanomaterial products for crop nutrition is increasing [3,4]. And some studies reported the application of Se fertilizers as mentioned by Mastronardi et al., [5]. Noteworthy to mention, the patent by [6] revealed new results, in which Nano-Se was supplemented to the soil during the plant growth cycle three to five times resulting in higher quality yield with a longer storage period of the blueberry crop. It was anticipated to join nanotechnology in the fertilizer industry [4] for increasing crop yield and achieving the highest agricultural productivity leading to food security from this perspective, considerable potentialities for the involvement of nanotechnologies for improving and developing the field of plant nutrition and plant fertilizers [5]. A number of research studies have proven that Se, even at low concentrations, can stimulate plant boom. It turned into discovered that 5 µg of Se stimulated a root boom and multiplied relative water content material in warm pepper vegetation by 13% in comparison to govern vegetation [7]. Furthermore, each of the concentrations of 3 and 5 µM Se stimulated a 25% spike in leaf area, which led to a boom inside the plant's common boom and biomass.

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Moreover, the boom of rice turned out to be inspired by the use of a low dose of Se. In 2019, Rady *et al.* [8] showed that tomatoes dealt at 40 M of Se hooked up multiplied drought tolerance, which turned into observed through numerous antioxidant enzymes actions, such as ascorbate peroxidase (APX) through 44%, superoxide dismutase (SOD) through 56%, and catalase (CAT) through 57%, respectively. On the other hand, medicinally it was remarked that the fertilization of plants with nano fertilizers led to increasing the secondary metabolites, and pigments which conferring the elevation of the antioxidant capacity and induction of cancer apoptosis. This study is concentrated on recent information related to SeNPs effects on in vitro derived *Opuntia ficus-indica* L. Mill concerning growth rate and the accumulation of secondary products and subsequently their effects on developing the lung anticancer activity in the *in vitro* bioassay evaluation.

2. EXPERIMENTAL

2.1 Plant material, Sterilization and germination

The Agriculture Research Center in Egypt provided seeds of cactus fruit (*Opuntia ficus-indica* L. Mill). The seeds were sterilized by washing them thoroughly with running tap water and then shaking them with 20% sodium hypochlorite and Tween 20% (polyoxyethylene sorbitol) for 10 minutes. Finally, they were washed three times with sterilized distilled water. According to our previous study by *Alaa et al.* [9], the best treatment for germination was applied to break the dormancy of cactus seeds, which resulted in the highest germination rate of 66%. All seeds were cultured on a half-strength MS basal medium supplemented with 3 % (w/v) sucrose, solidified with 0.8 % (w/v) agar. After adjusting the pH to 5.8, aseptic plantlets were grown after incubating at 25 °C 2 °C for 8 weeks.

2.2 Micropropagtion

The germinated shoots of plants were micro propagated by growing them on full-strength MS basal medium that was supplemented with 3 % (w/v) sucrose and solidified with 0.8 % (w/v) agar. After adjusting the pH to 5.8, the incubation was conducted at 25°C 2°C, and the plants were obtained after 6 weeks.

2.3 Preparation of Selenium Nanoparticles

Selenium nanoparticle solutions at two different concentrations (2.5 and 5 mg l^{-1}) were obtained through personal communication with Professor Osama Darwish. Both solutions have been previously prepared by Darwish *et al.* [10]. Stock solutions of both 100 mM sodium selenite and 50 mM ascorbic acid were prepared using sterile deionized water. Ascorbic acid was mixed dropwise with the prepared sodium selenite solution under magnetic stirring (600 rpm) at room temperature such that the final ratio of sodium selenite to ascorbic acid was 1:4. The mixture was allowed to react until the colour changed from colorless to bright orange. The prepared selenium nanoparticles were characterized by TEM analysis, revealing a spherical shape form with a narrow range of particle size distribution and with 15-35 nm for SeNPs size. TEM micrograph analysis demonstrated that the mean size of the prepared selenium nanoparticles was comparable to the particle size that has been reported in the study by Srivastava *et al.* [11].

2.4. Effect of the synthesized selenium nanoparticles on in-vitro derived plants

To investigate the effects of Selenium nanoparticle (SNP) treatments, plants were randomly selected and cultured using either MS basal medium (control) or full strength MS basal medium (full strength). The basal medium was supplemented with 3% sucrose and solidified using 0.8% agar, then supplemented with two different concentrations (2.5% and 5% l-1) of Selenium nanoparticles. The pH of the medium was set at 5.8. After 6 weeks, the plants were incubated at 25 °C ± 2 °C.

2.5 Preparation of samples and determination of total phenolics

In order to prepare samples for analysis and determine total phenolic contents, the *in vitro* derived cladodes of the sample were delicately cut into smaller pieces and subjected to a 3-day drying process in an oven heated to 40°C. Subsequently, the fragments of the cladode were ground for a period of 3 minutes in an internal coffee grinder before being sieved. Finally, the total phenolic contents were determined on all extracts using the folin-polyphenolic method, as previously described in [12].

2.6 Determination of the cytotoxicity of in-vitro derived cladode extracts on cells using the MTT protocol

This study investigated the potential for in vitro-derived cladode extracts to be cytotoxic to cells using the Methods for Therapeutic Toxicity (MTT) protocol. An ethanol extract, which contains phenolic and other bioactive compounds, was used to evaluate the anticancer potential of the extracted compounds. To further investigate the efficacy of the extracted compounds against lung cancer, an A549 lung cell sample was used in accordance with the Metabolic Testing Protocol (MTT) [13]. The experiment was repeated three times for statistical analysis purposes.

2.7 Statistical Analysis

The experimental design used consisted of randomized complete blocks with three replications. Statistical analyses were performed using program IBM® SPSS® (SPSS Inc, IBM Corporation, NY, USA) Statistics Version 25 (2017) for Windows. Data were subjected to ANOVA, and P values <0.05 were considered statistically significant. Treatment means were compared using the least significant difference post hoc test reported in [14]. A P value of <0.05 is considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effects of nano-selenium treatments on the in-vitro cladodes of Opuntia ficus-indica L. Mill

In a limited number of studies, SeNPs have been compared to other inorganic forms of Se as a fertilizer in higher plants, such as rice, tomato, giant reed, and tobacco [15-18]. The results of these studies indicated that nano-Se had a distinct biological and physiological effect on these plants, with real differences between the two. It is observed that nano-Se, at a concentration extent of 50–100mg kg-1, significantly stimulated organogenesis and root system growth, while selenite, at any concentration, inhibited both callus growth and root regeneration. Furthermore, nano-Se concentrations were found to stimulate the initiation and extension of roots, as well as the production of biomass [19]. Regarding this study, the examined treatments of Selenium nanoparticles solutions at two different concentrations 2.5 and 5 mg l^{-1} differed in their effects on the shoot length and the number of shoots per explant showing the highest number of shoots (11.3±0.33) with the addition of 5.0 $mg1^{-1}$ of SeNPs treatment to full MS medium compared to the addition of 2.5 $mg1^{-1}$ of SeNPs treatment which showed 6.0 ±0.5 shoots per explant and the least number of shoots 3.0±0.5 with the medium of control when the plants cultured on the full MS medium without any addition. Moreover, the addition of 0.5 $mg1^{-1}$ of SeNPs treatment led to an increase in the shoot length to show the highest shoot length at 8.20±0.47 cm compared to the addition of 2.5 $mg1^{-1}$ of SeNPs treatment at 5.73±0.20 cm and the lowest shoot length at 3.2±0.49 cm with the medium of control when the plants cultured on the full MS medium without any addition. These promising results reveal for the first time the role of SeNPs treatments in the multiplication stage of *in-vitro*-derived plants of *Opuntia ficus-indica L.* Mill without any addition of plant growth regulators.

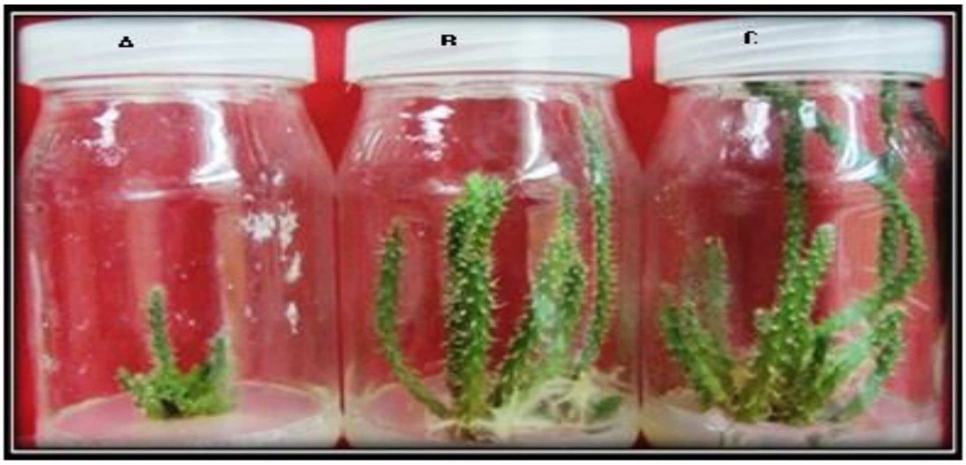

Figure 1 Effects of selenium nanoparticles SeNPs) on *Opuntia* explants, (a) control, (b) 2.5 and (c) 5 mg l^{-1} on the rate of multiplication (number of shoots) and shoot length

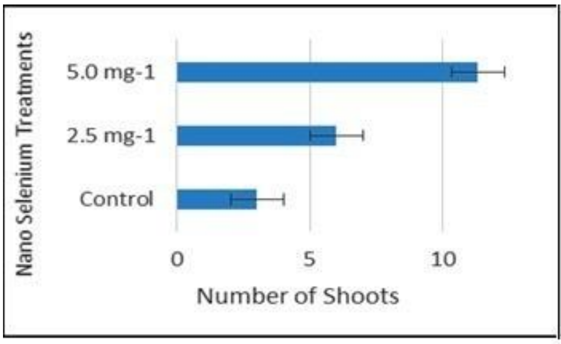

Figure 2 Effects of the treatments of selenium nanoparticles at 2.5 and 5 mg l-1 on number of shoots compared with control without SeNPs treatment.

More specifically, it has been found that selenium can facilitate stress in plants since it causes the production of secondary metabolites and increases the activities of most antioxidant enzymes. Remarkably in this study, the treatment with SeNPs enhanced the increase of total phenolics, and that increased more with the higher concentration of SeNPs treatment at 5.0 mg1-1 as shown in fig 4, the total phenolic increased to 168.5 mg/g and less with the SeNPs treatment at 2.5 mg1-1recording 91.5 mg/g compared to the control sample without SeNPs treatment which recorded 86 mg/g. The previous study on broccoli sprout reported that the supplying of 50 and 100 mg/L SeNPs increased the total phenols content of roots compared with the control and significantly increased the antioxidant capacity of shoots in all treated samples with SeNPs about the roots, significantly increased the antioxidant capacity with 10 mg/l of the treatment [20]. These recorded results refer to the important perspective for applying selenium nanoparticles in future studies targeting the stimulation of the secondary metabolites in cactus plant.

3.3 Evaluation of the activity of in-vitro cladode extracts on the viability and the proliferation/migration of A549 cells

For the bioassay evaluation of the extracts from *in-vitro* derived cladodes, the A549 cells were used as a specific model to assess the produced bioactive compounds as potential anticancer agents. The effects of the extract of sample 1, *in-vitro* cladodes, were previously treated with the higher concentration of SeNPs at 5.0 mg 1-1 on A549 cells and were tested by MTT assay. The A549 cells were treated with various concentrations of the extract as recorded in Table 1; the inhibition of the growth for A549 was dose-dependent as shown in Figure 6, with an IC50 of 3535.92 µg/ml. The extracts of control plant sample 2, *in vitro* cladodes, were cultured on full MS basal medium without SeNPs treatment and the MTT protocol was carried out at the same concentrations as shown in Table 1. The crude extract of sample 2 has a proliferative effect of on A549 cells. The result exhibited the growth inhibition of A549, based on dose-dependent, with an IC50 of 3766.16 µg/ml and showed an anticancer activity of the produced extracts (Figure 7). Recently, more research results confirmed that various compounds such as phenolic, different coloured pigments, and flavonoids have considerable antioxidant activity which influences significantly the prevention of

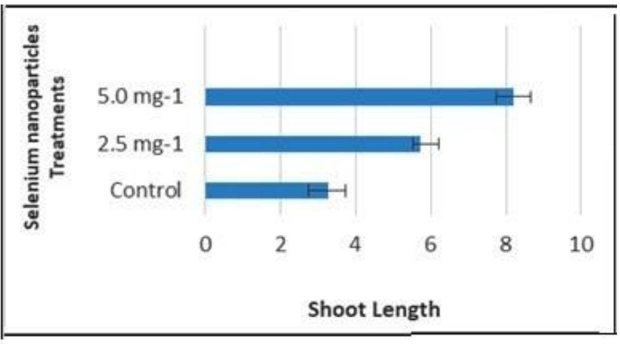

Figure 3 Effects of the treatments of selenium nanoparticles at 2.5 and 5 mg l-1 on shoot length compared with control without SeNPs treatment.

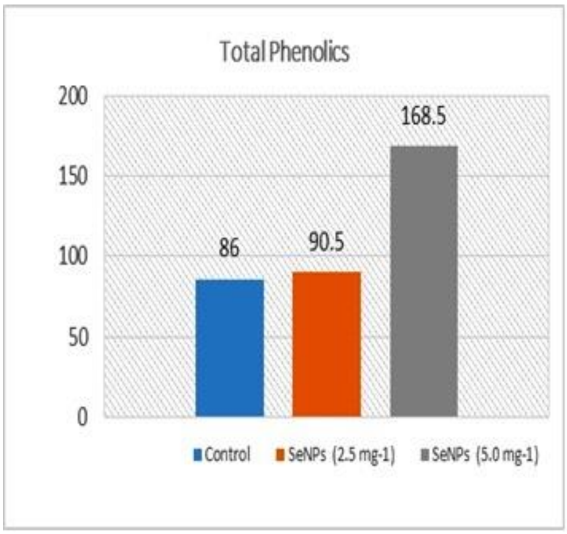

Figure 4 Effects of different treatments of selenium nanoparticles on the accumulation of total phenolic compared to the control In-vitro cladode extract without SeNPs.

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Table 1 The effects of different concentrations of *in-vitro* cladode [treated with SeNPs (1) and untreated (2)] extracts on both the viability and proliferation/migration of (lung cancer) A549

ID	Conc. ug/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
A549	1:2	0.315	0.309	0.3	0.308	0.004359	100	0	ug/ml
1	10000	0.024	0.023	0.03	0.025667	0.002186	8.333333333	91.66666667	3535.92
	5000	0.051	0.068	0.072	0.063667	0.006438	20.67099567	79.32900433	
	2500	0.225	0.201	0.211	0.212333	0.00696	68.93939394	31.06060606	
	1250	0.296	0.314	0.302	0.304	0.005292	98.7012987	1.298701299	
	625	0.307	0.299	0.309	0.305	0.003055	99.02597403	0.974025974	
	312.5	0.313	0.303	0.308	0.308	0.002887	100	0	
2	10000	0.022	0.035	0.024	0.027	0.004041	8.766233766	91.23376623	3766.16
	5000	0.06	0.084	0.089	0.077667	0.00895	25.21645022	74.78354978	
	2500	0.236	0.241	0.228	0.235	0.003786	76.2987013	23.7012987	
	1250	0.309	0.299	0.307	0.305	0.003055	99.02597403	0.974025974	
	625	0.323	0.297	0.301	0.307	0.008083	99.67532468	0.324675325	
	312.5	0.298	0.324	0.31	0.310667	0.007513	100.8658009	0	

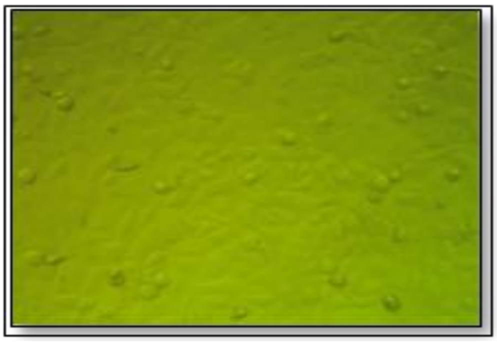

Figure 5. Control A549 cells, Organism: *homo sapiens*, human, Tissue: lung, Cell Type:, epithelial, Culture, Properties: adherent, Disease: Carcinoma

chronic diseases, specifically cancer [21]. Furthermore, the reduction of the risk of different cancers (liver, prostate, mammary, colorectal, skin, and lung) was shown to be related to the intake of selenium element [22]. This study is deemed to be the first that provides the effects of SeNPs treatment on the enhancement of the production of bioactive compounds in *in-vitro* plants and estimates their anticancer activities against one of the common cancers in our country Egypt lung cancer.

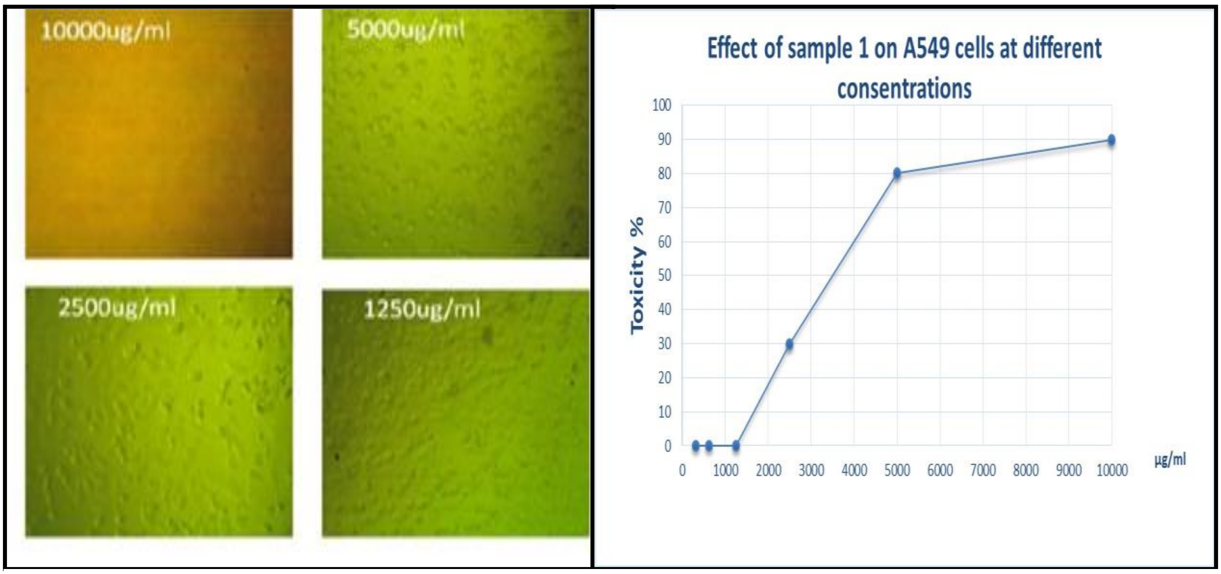

Figure 6 The effect of sample 1 (extract produced from treated in vitro cladodes with SeNPs at 5.0 mg/l) on the A549 cells (Lung cancer cells).

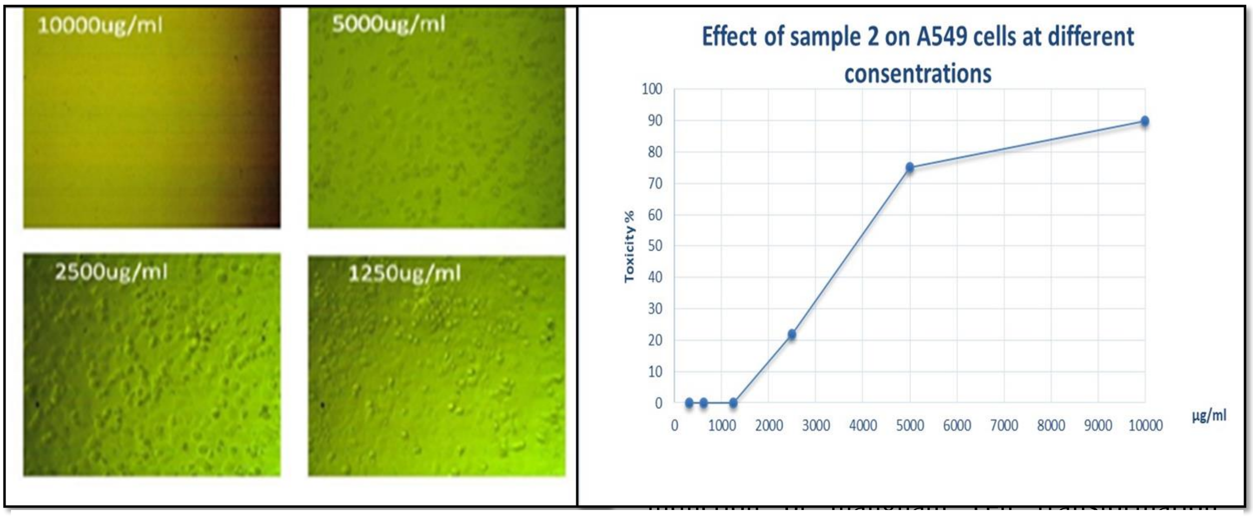

Figure 7 The effect of sample 2 (extract of *in-vitro* cladode without SeNPs on A549 cells (Lung cancer cells)

Another study by Tiloke *et al.* [23] revealed that plants exhibited anti-cancer potential by interfering with signaling cascades that increase the proliferation of cancer cells. Inhibition of cancer cell viability may be due to phenolic compounds [24]. The decreased cell viability in Hela cancer cells may be caused by the solvent fraction of *Moringa* oleifera leaves, considering its ability to cause increased apoptosis through downregulation of E2F1(E2 promoter binding factor 1)and up regulation of Bax protein. Based on previous data, the polyphenol-rich sap of various prickly pear cacti was shown to affect the HT-29 colon cancer cell line by inducing cycle arrest [25]. A profound insight was gained that this effect is caused by an increase in ROS leading to cell death, and the same effect was also reported in ovarian cancer cells [26]. One of the most insightful studies of *in vitro*-derived cladodes of prickly pear species was the study of callus and cell suspensions of three prickly pear species (*Opuntia streptacantha*, *Opuntiamegacantha*, and *Opuntiaficus-indica*). They estimated the total accumulation of phenolic acids and flavonoids then reported their contents. Although phenolic compounds, flavonoids, and antioxidant activities show similarities in callus and suspension systems, 1.5- to 1.9-fold higher concentrations of antioxidant compounds accumulate *in vivo* twigs compared to control sample results [27]. It was demonstrated that*invitro* culture of *Opuntia*is an effective system for obtaining the metabolites of these species. The findings of our earlier research align with our current study and found the highest estimation of total

significantly. Otherwise, this novel investigation applies an advanced protocol for enhancing the production of total phenolics by adding SeNPsat a rate of 5.0 mg1^{-1}, which increases the maximum estimate of 168.5 mg/g, and thus improves the viability and proliferation /migration of A549 lung cells, thereby detecting another natural agent for lung cancer prevention and treatment. Interestingly, the phenolic compound's existence in *Moringa oleifera* leaves may reduce cell viability by inhibiting the cancer survival pathway and NFK (nuclear factor kappa-B) signalling pathway through down regulation of p65 component. A study on *Moringa oleifera* leaves revealed that increasing the total phenolics in leaves could induce apoptosis in lung cancer [28], which was consistent with the discovery of novel anticancer agents.

CONCLUSION

This study reveals the potential of using the *in-vitro* cladode extract of Egyptian prickly pear (produced under selenium nano *in-vitro* protocol) as an anticancer agent. The highest micro propagation rate of 11.33 Shoot /explant was recorded through an effective protocol using SeNPs at a concentration of 5.0 mg l-1 and a maximum shoot length of 8.20 cm. Results

ETHICAL APPROVAL

Experiments were carried out according to Ethical Committee of Medical Research of the National Research Centre, Egypt with approval no. 14478092023

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